Lipofection of Cas9/synthetic RNA ribonucleoprotein (RNP) complexes for CRISPR/Cas9 genome editing (Thermo CRISPRMAX™ Kit)

BACKGROUND

This protocol describes how to transfect cultured cells with ribonucleoprotein (RNP) complexes that consist of purified Cas9 nuclease duplexed with synthetic guide RNA (gRNA; synthetic sgRNA or annealed crRNA and tracrRNA) using the Lipofectamine™ CRISPRMAX™ Transfection kit. Delivery of RNPs means that CRISPR components exist only transiently inside the cell, limiting Cas9 and guide RNA expression – this allows for the highest levels of editing efficiency and greatly reduces the chances of possible off-target and toxic effects. Furthermore, the use of synthetic guide RNAs eliminates the risk of incorporating foreign DNA into the host genome, which can occur when using plasmid-based guides. Synthego synthetic guide RNAs are of the highest quality and offer a superior alternative to *in vitro* transcribed (IVT) guide RNAs that are of variable quality and produce inconsistent editing results.

MATERIALS REQUIRED

Reagent/Material	Vendor
Synthego CRISPRevolution synthetic guide RNA (sgRNA or crRNA/tracrRNA)	Synthego
Synthego 2NLS-Cas9 nuclease	Synthego
Opti-MEM™ I Reduced Serum Medium	Thermo Fisher (Catalog #31985)
Lipofectamine™ CRISPRMAX™ Transfection Reagent with Cas9 PLUS™ Reagent	Thermo Fisher (Catalog #CMAX00001 and variants)
Sterile tissue culture plates (e.g., 96-well, 24-well etc.)	Common lab supplier
Microcentrifuge tubes	Common lab supplier

IMPORTANT CONSIDERATIONS

- All Synthego and CRISPRMAX reagents should be stored according to the manufacturer's recommendations.
- This protocol is optimized for use with commonly used cell lines, such as HEK293T, A549, U2OS, HeLa, CHO, MCF-7. It may be necessary to experimentally optimize this protocol for a particular cell line.
- Successful transfection is critically dependent on cell density. It may be necessary to experimentally optimize cell seeding densities in order to determine the most appropriate level of confluence for transfection.
- For fast growing cells, seed fewer cells. Cell seeding is based on the rate of cell growth. Suggested starting cell numbers are listed in the protocol below.
- Use cells at lowest passage number possible.
- Please follow the colored text guide for suggested volumes for each plate volume:

(96-well / 24-well / 6-well)

- Synthetic guide RNA (sgRNA or crRNA/tracrRNA) should be dissolved in an appropriate buffer and diluted to a working concentration using nuclease-free water. If using the two-component system of crRNA/tracrRNA, these should be annealed together beforehand. Please consult the Synthego Quick Start Guide for best practices related to dissolving, annealing and storing synthetic guide RNAs.
- Synthego Cas9 nuclease (20pmol/µl) can be diluted in Opti-MEM™ I Reduced Serum Medium in order to achieve a working concentration for each plate volume.
- RNP complexes are stable at room temperature for up to 2 hours, and at 4°C for several weeks. Note that RNPs stored at 4°C may become susceptible to contamination from microbial growth after long periods of time.
- RNP complexes are formed in Opti-MEM™ I Reduced Serum Medium and can be added directly to cells in culture medium irrespective of antibiotics. Following transfection, it is not necessary to remove RNP complexes or to add or change media.
- Always maintain sterile technique, and use sterile, filter micropippetor tips.

TIME CONSIDERATIONS

- Growth of cells for transfection (before transfection): ~ 2-3 days
- Preparation of RNPs and reagents: ~ 15 minutes
- Incubation with cells: ~ 5 minutes, 10 minutes
- Final incubation (after transfection): ~ 2-3 days

EXPERIMENTAL OUTLINE

1. Seed cells (96-well / 24-well / 6-well) so that they are 30-70% confluent for transfection.
2. Prepare RNP complexes (synthetic guide RNA + Cas9 nuclease) in transfection reagents.
3. Add the RNP complex-transfection mixture to cells.
4. Incubate for 2-3 days.
5. Perform an appropriate genomic editing assay (e.g., PCR followed by genomic cleavage detection and/or TIDE anlaysis; Next-Gen Sequencing)

PROTOCOL

Please Note

It is critical to add reagents in the order recommended below. For amounts of Cas9 nuclease and synthetic guide RNA, a recommended range is given; it may be necessary to experimentally optimize these volumes and ratios for RNP formation for each cell type. Prepare the RNP complexes with the Lipofectamine™ Cas9 Plus™ Reagent and Opti-MEM™ I Reduced Serum Medium in a separate tube before adding into diluted CRISPRMAX™ Reagent.

Reaction volumes (96-well / 24-well / 6-well) are for EACH WELL and should be scaled up proportionally for the number of wells to be used.

Day 0

Seed cells so that they are 30-70% confluent for transfection

For 96-well culture, expect to start with 0.7 – 2 x 10^4 cells/well and a volume of 100µl growth medium/well.

For 24-well culture, expect to start with 0.42 – 1.2 x 10^5 cells/well and a volume of 500µl growth medium/well.

For 6-well culture, expect to start with 2.1 – 6 x 10^5 cells/well and a volume of 2ml growth medium/well.

PROTOCOL (continued)

Day 1

1. **Prepare RNPs in a microfuge tube:**

   For 96-well culture, mix:
   1pmol of Cas9 nuclease (range: 0.5 – 5pmol)*
   1pmol of guide RNA (sgRNA or annealed crRNA:tracrRNA; range: 0.5 – 5pmol)*
   0.17µl of Lipofectamine™ Cas9 Plus™ Reagent
   5µl Opti-MEM™ I Reduced Serum Medium

   For 24-well culture, mix:
   10pmol of Cas9 nuclease (range: 1 – 20pmol)*
   10pmol of guide RNA (sgRNA or annealed crRNA:tracrRNA; range: 1 – 20pmol)*
   1µl of Lipofectamine™ Cas9 Plus™ Reagent
   25µl Opti-MEM™ I Reduced Serum Medium

   For 6-well culture, mix:
   20pmol of Cas9 nuclease (range: 10 – 30pmol)*
   20pmol of guide RNA (sgRNA or annealed crRNA:tracrRNA; range: 10 – 30pmol)*
   5µl of Lipofectamine™ Cas9 Plus™ Reagent
   125µl Opti-MEM™ I Reduced Serum Medium

* You may need to experimentally determine the optimum amounts of Cas9 nuclease and guide RNA. In general, a 1:1 ratio of Cas9 nuclease:guide RNA is suggested for RNP formation.

2. **Incubate RNPs for 5 minutes at room temperature.**

3. **In a separate tube, dilute CRISPRMAX™ Reagent in Opti-MEM™ I Reduced Serum Medium:**

   For 96-well culture, mix:
   5µl Opti-MEM™ I Reduced Serum Medium
   0.3µl Lipofectamine™ CRISPRMAX™ Transfection Reagent

   For 24-well culture, mix:
   25µl Opti-MEM™ I Reduced Serum Medium
   1.5µl Lipofectamine™ CRISPRMAX™ Transfection Reagent

PROTOCOL (continued)

For 6-well culture, mix:
125µl Opti-MEM™ I Reduced Serum Medium
7.5µl Lipofectamine™ CRISPRMAX™ Transfection Reagent

4. **Add the RNP complex mixtures directly to the diluted Lipofectamine™ CRISPRMAX™ Transfection Reagent tube, and mix well by pipetting up and down.**

5. **Incubate for 5-10 minutes at room temperature. Do need exceed 30 minutes.**

6. **Add the RNP complex-CRISPRMAX mixture to cells:**

   For 96-well culture, add 10µl (each well)

   For 24-well culture, add 50µl (each well)

   For 6-well culture, add 250µl (each well)

7. **Incubate cells for 2-3 days at 37°C.**

Days 2-4

Visualize and analyze transfected cells:

Rinse cells with 50-500µl PBS. Analyze an aliquot of cells by lysing cells with 20-250µl lysis buffer, perform PCR of region(s) of interest and perform genomic cleavage assay and/or TIDE analysis.

REFERENCE

Thermo CRISPRMAX Kit

Find out more at synthego.com

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